AD________________

Award Number: DAMD17-00-1-0036

TITLE: The Role of aVB6-mediated latent TGF-B Activation in Prostate Cancer

PRINCIPAL INVESTIGATOR: John S. Munger, M.D.

CONTRACTING ORGANIZATION: New York University School of Medicine
New York City, New York 10016

REPORT DATE: January 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

20010723 109

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE
January 2001

3. REPORT TYPE AND DATES COVERED
Annual (1 Jan 00 - 31 Dec 00)

4. TITLE AND SUBTITLE
The Role of aVB6-mediated latent TGF-B Activation in Prostate Cancer

5. FUNDING NUMBERS
DAMD17-00-1-0036

6. AUTHOR(S)
John S. Munger, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
New York Universitv School of Medicine
New York City, New York 10016

E-Mail: mungej01@med.nyu.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Abundant evidence suggests that overexpression of TGF-ß by prostate cancer cells enhances their ability to grow and metastasize. TGF-ß is secreted by cells in a latent form that results from a noncovalent interaction between TGF-ß and its propeptide (latency-associated peptide, LAP). Mechanisms leading to active TGF-ß are poorly understood at present. Our lab discovered a mechanism of TGF-ß activation in which the integrin αVß6 binds to an RGD sequence near the C-terminus of LAP. αVß6 is only expressed in epithelial cells. We hypothesize that αVß6, by activating TGF-ß1, is an important regulator of normal prostate epithelial proliferation, and that overexpression of aVb6 by prostate tumor cells acts in concert with overexpression of its ligand latent TGF-ß1 to produce active TGF-ß1 and promote growth of the tumor. In this work, we are testing whether the ß6 integrin subunit is regulated by androgen, whether it is overexpressed in human prostate cancer, and whether it affects growth and metastasis of prostate cancer in an animal model. Our results to date indicate that ß6 is expressed in prostate luminal epithelial cells, which activate TGFß1 in a ß6-dependent manner, and that ß6 expression is upregulated in the mouse in a delayed fashion after castration.

14. SUBJECT TERMS
transforming frowth factor-B, integrin, TGFB activation, PTEN, phosphatase, cell proliferation, epithelium

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The subject of this work is a system for the activation of latent TGFß in the prostate. The system consists of the $\alpha V\beta 6$ integrin expressed on epithelial cells. Our previous work showed that this integrin can bind to an integrin recognition site (arg-gly-asp) on latent TGFß1 and effect its activation [1]. TGFß is known to be important for regulating the growth and differentiation of various epithelia, and also to be important in cancer growth. Little is currently known about this system in the prostate: eg, what cells express $\alpha V\beta 6$, how expression of the integrin is regulated, and if and when this system regulates prostate epithelial growth via production of active TGFß1. The purpose of the work is to demonstrate whether or not this system plays a role in prostate cancer. The scope of the work involves cell line and mouse experiments (to gauge the normal expression and regulation of $\alpha V\beta 6$ in the prostate), and evaluation of human prostate cancer tissue and an *in vivo* mouse prostate cancer model (to address the question, does $\alpha V\beta 6$-mediated activation of TGFß1 promote prostate cancer growth?).

BODY

The first 12 months of the project addressed tasks 1 (determine ß6 expression in normal prostate and prostate cancer cells) and 2 (determine the effect of androgen on ß6 expression).

We began by looking at well-established prostate cell lines established by Dr. EL Wilson of NYU [2]. One line has characteristics of prostate luminal epithelial (LE) cells, the other of prostate basal epithelial (BE) cells. Because the ß6 integrin is expressed only in epithelial cells, we did not look at other cell types (e.g., prostate smooth muscle cells).

We did immunostaining using a rabbit monoclonal Ab (designated B1) obtained from D Sheppard at UCSF. We found that the LE cells stained intensely for ß6 integrin, whereas the BE cells were negative. We confirmed these results functionally in 2 ways. First, we did adhesion assays using recombinant LAP-ß1, the ligand for $\alpha V\beta 6$. LE cells, but not BE cells, were able to adhere and spread on plastic coated with LAP-ß1 at coating concentrations similar to that needed for other cell types expressing ß6 integrin (e.g., transfected SW-480 colon carcinoma cells). Second, we found that LE cells in culture were able to activate TGFß1 in an $\alpha V\beta 6$-dependent manner. This is shown in Figure 1.

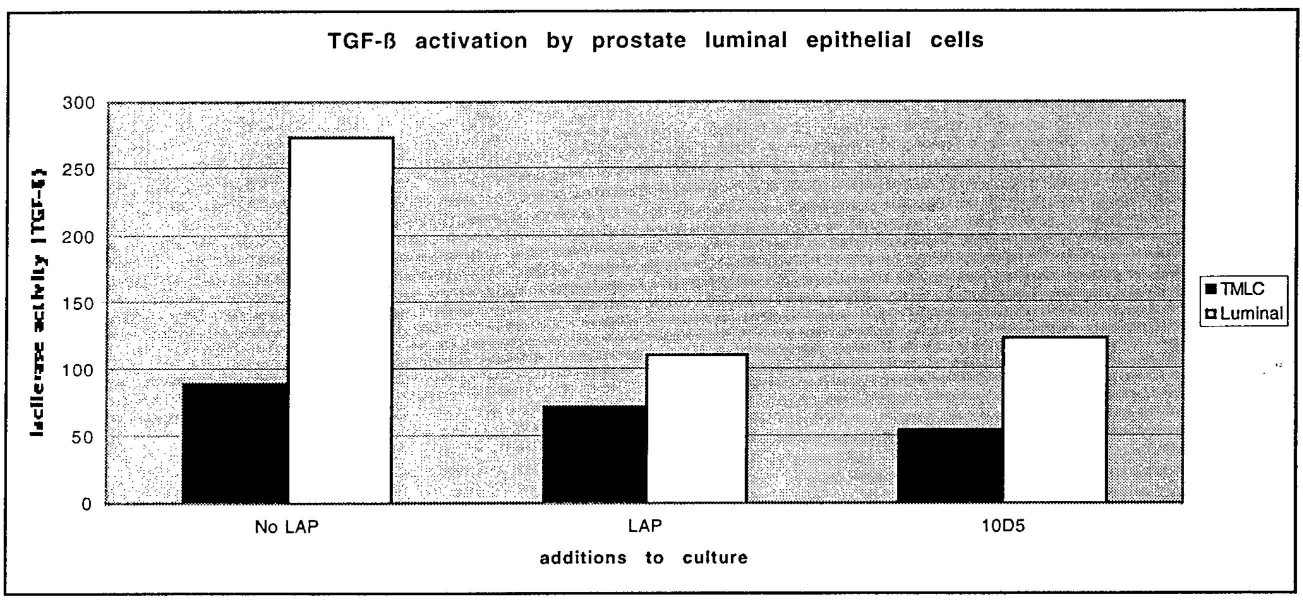

Figure 1. LE cells were cocultured with reporter cells (TMLC) that respond to TGF-ß by producing luciferase. Compared to reporter cells cultured alone (black bars), reporter cells cultured with LE cells detect active TGFß (white bars); the signal is abrogated by a reagent that inhibits active TGFß (LAP) and by an anti-ß6 Ab (10D5), demonstrating that the active TGFß is produced via $\alpha V\beta 6$.

We next examined the effect of androgen on ß6 expression by these cells. Both cell types express androgen receptor (AR), and both cell types are normally cultured in the presence of testosterone. Dihydrotestosterone (DHT) is added to the serum-free culture medium of the LE cells, and is present in the 10% serum included in the BE cell medium. Therefore, we set up subconfluent cultures of LE and BE cells in medium without DHT (for the BE cells this was done by using charcoal-treated serum). After DHT-free growth for 24 or 48 hours, cells were immunostained for ß6 integrin. The staining patterns were unchanged: LE cells still expressed a high level of ß6 and BE cells were negative. Thus, at least in cell culture, we find no evidence of androgen regulation of ß6 expression.

We also examined TGFß activation by LE and BE cells in the presence and absence of DHT. As expected, based on the immunostaining results, the BE cells did not activate TGFß, regardless of DHT status. However, when LE cells are cultured for 24 h in the absence of DHT and then cocultured with TGFß-sensitive reporter cells, we find increased active TGFß. This is shown in Figure 2.

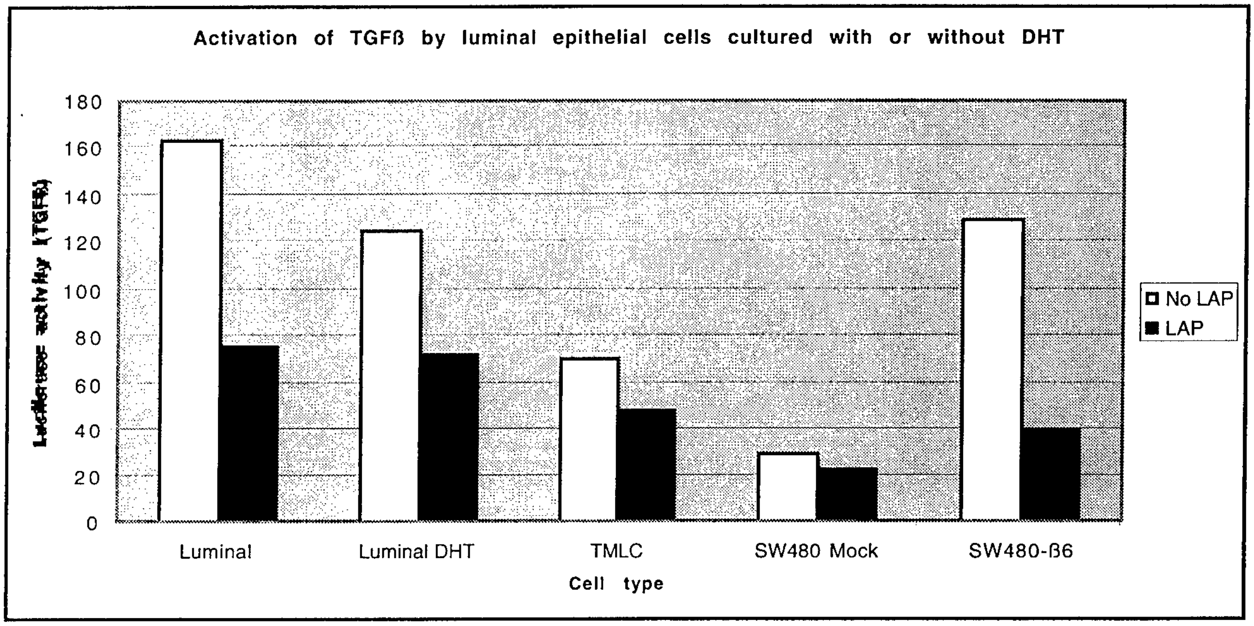

Figure 2. Luminal epithelial cells were cultured with or without DHT for 24 h, then cocultured for an additional 24 hours with TGFß-responsive reporter cells to measure production of active TGFß. For each condition, cells were cultured with and without a reagent that inhibits active TGFß (LAP); in each case, the difference between luciferase activity with and without LAP is proportional to the amount of active TGFß. SW480 colon Ca cells mock- or ß6-transfected are shown as negative and positive controls for TGFß activation, respectively.

Thus, in short-term cell culture experiments we find that ß6 expression is not androgen-regulated, although the ß6-expressing LE cells are able to activate greater amounts of TGFß in the absence of DHT. This latter effect might be due to increased TGFß expression or to some other effect, such as enhanced interaction of $\alpha V\beta 6$ with the cytoskeleton.

We have also looked at the regulation of ß6 within the murine prostate as a function of androgen. We do this by immunostaining frozen sections of mouse prostate obtained from normal or castrated mice. In normal mouse prostate, we find minimal ß6 expression in prostate epithelium. Similarly, in mice that are 2 days and 5 days post-castration we detect only trace amounts of epithelial ß6 expression. However, when we examined prostates from mice 8 weeks post-castration, we found high levels of ß6 expression in prostate epithelium. ß6 was clearly expressed in luminal cells; in addition, in many areas there was increased staining adjacent to the basement membrane that might represent ß6-expressing basal cells.

Thus, our *in vivo* data suggest that prostate ß6 expression is affected by androgen, but in a delayed and therefore perhaps indirect manner. One can speculate that active TGFß generated by αVβ6 in the involuted prostate contributes to the growth arrest observed in that state.

An obvious test of this idea is to examine ß6 knockout mice after castration. This is planned as part of task 3 in the coming year. In anticipation of this, we have acquired the ß6 KO mice (on a C57Bl/6 background). Because these mice will also be crossed with a transgenic mouse line (TRAMP mice, which develop prostate cancer), we plan to house the ß6 mice in our transgenic facility. Doing so requires that the mice be rederived (superovulated WT females are impregnated by ß6 KO males in another facility; the fertilized eggs are removed and implanted in a recipient female within the transgenic facility). Therefore, over the last 4 months we have been working to generate these rederived mice and currently have 5 heterozygous pups and two more pregnant recipient females. Once these mice are available in sufficient numbers, we will examine the histology of ß6 KO prostates and compare prostate proliferation (by BrdU labeling) in wild type and ß6 KO mice (in the normal state, during prostate involution after castration and then during prostate regeneration following DHT pellet implantation).

In other work, we have made a recent observation that may bear on this work. Work by S Nishimura (UCSF), in which our lab collaborated, has shown that the integrin αVβ8 can activate latent TGFß1. This has now been reported in abstract form at the ASCB meeting in December 2000, and a manuscript (on which I am an author) is in preparation. ß8 is expressed more widely than is ß6 - it is expressed not only in epithelial cells but also in smooth muscle cells and the CNS. There is no published work on ß8 expression in the prostate. The importance to this project is that ß8 is therefore another potential player as an activator of TGFß in the prostate. Currently I do not plan to modify the proposed work to examine ß8; however, if as we progress observations suggest a major role for ß8 in the prostate I may discuss a possible change/addition to our plan with the US Army Medical Research and Materiel Command staff.

KEY RESEARCH ACCOMPLISHMENTS

- In cell lines, $\alpha V\beta 6$ is expressed at high levels in luminal but not basal prostate epithelial cells.
- In short-term experiments, ß6 is not regulated by androgen in either the murine prostate or in cell lines. However, in long-term experiments, ß6 expression is upregulated in the involuted prostate of castrated mice. This is consistent with the idea that ß6-mediated TGFß activation is kept at low levels in the normal prostate, but is important in maintaining the involuted state (TGFß inhibits epithelial cell growth).
- Although androgen removal does not increase $\alpha V\beta 6$ expression in luminal prostate cell lines, it does increase TGFß activation.

REPORTABLE OUTCOMES

None yet. We need data from ß6 knockout mice (task 3, year 2) to complement the current results.

CONCLUSIONS

These experiments are the first to examine the role of αVß6-mediated activation of latent TGFß in the prostate. Our results suggest that normally αVß6 expression in the prostate epithelium is kept at a low level, which (we theorize) is one factor allowing normal expression of the prostate epithelium. Interestingly, there is a delayed expression of ß6 integrin after castration, which may contribute to the continued inhibition of epithelial growth in that state.

Our results also show that prostate cell lines (specifically, a luminal cell line) can express αVß6 and activate TGFß1 in an αVß6-dependent manner. Although this particular cell line is not tumorigenic, it is immortalized and p53-null and therefore presumably has some characteristics of prostate cancer cells. Work in the next 2 years will directly examine the expression and influence of αVß6 in malignant prostate cells.

The first year of this project has also been important as a training period in the techniques needed for analyzing murine prostate. We are now in a strong position to extend the work to the subsequent tasks.

"So what." There are many reports in the literature relating cancer outcomes and tumor cell behavior to increased expression of TGFß by tumor cells. However, to my knowledge there has never been an analysis of the role of a TGFß activator in tumor cells. Yet, our results with αVβ6 and lung fibrosis [1] point out the critical role that a TGFß activator can play in a TGFß-dependent process. If a specific TGFß activator can be identified as important in a cancer, this knowledge might be important for determining prognosis and for developing therapies in which the activator is a target.

REFERENCES

1. Munger, J.S., et al., *The integrin $\alpha v\beta 6$ binds and activates latent TGFß1: a mechanism for regulating pulmonary inflammation and fibrosis*. Cell, 1999. **96**: p. 319-328.
2. Salm, S., et al., *Generation of active TGF-ß by prostatic cell cocultures using novel basal and luminal prostatic epithelial cell lines*. Journal of Cellular Physiology, 2000. **184**: p. 70-79.